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Educated at Cambridge University (BA in Natural Sciences, 1957) and University of California at Davis (PhD in Biochemistry and Biophysics, 1961). Member of Faculty of the School of Medicine at the University of Pennsylvania since 1961, Professor of Biochemistry and Biophysics since 1972 (twice chairman) and Emeritus Professor since 1988. Guggenheim Fellow (Oxford, University College London, and Bristol) in 1971, Fogarty Senior International Fellow (Bristol) in 1979, Sabbatical at the Open University in 1987. Eighty refereed papers in mechanistic enzymology, stopped flow kinetics. ONR Program Officer since 1988 in area of Molecular Biomimetics.

Green Synthesis of Energetic Materials

This small and relatively new ONR/SERDP-funded program demonstrates how the unsurpassed regio-, stereo- and reaction-specificity and efficiency of enzymes (and of biomimetic catalysts), resulting from the natural evolution of molecular recognition strategies over aeons, may be harnessed and further developed for the “green” synthesis of energetic materials (EM) of DoD and commercial interest. Each of the target materials, namely, 2,3-dimethyl-2,3-dinitrobutane (DNDMB, a taggant), 2,2',4,4',6,6'-hexanitrostilbene (HNS, a heat-resistant EM) and 1,2,4-butanetriol (BT, an EM precursor and plasticizer) is in current DoD use and the enzyme or biomimetic catalyst in each case replaces a simple, conventional, synthetic chemical scheme. The DNDMB work, involving peroxidative free radical coupling catalyzed by off-the-shelf (wild type, or WT) peroxidase, has reached the scale-up stage. The HNS synthesis postulates peroxidative halogenation of the methyl group of DNT/TNT by WT haloperoxidases. This has not yet been demonstrated, although novel ring halogenation by WT soybean peroxidase is observed. The route chosen for BT is hydroxylation by dioxygen of 1,4-butanediol catalyzed by a biomimetic high potential iron porphyrin. A significant breakthrough in catalyst development is reported, but hydroxylation has not yet been attempted. Peroxidases and hydroxylases are being engineered in a parallel ONR program, using in particular directed enzyme evolution, to have improved properties (compared to WT, eg thermal and oxidative stability) for enzymatic processing of EM.

GREEN ENERGETICS

DEFENSE S&T SEMINAR ON EMERGING TECHNOLOGIES

June 18, 1999

BIOCATALYSTS FOR ENERGETIC MATERIALS SYNTHESIS

Biotechnological research to provide proof of principle
for application and preliminary scale-up of enzymes
and biomimetic catalysts in energetic materials processing

Dr. Harold J. Bright

Molecular Biomimetics Program

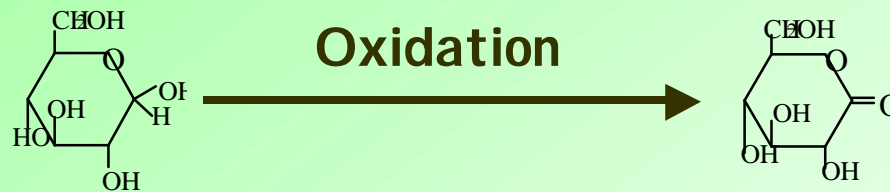
ONR 335

OUTLINE OF BRIEF

1. Conventional vs. Green Processing
2. EM targets
 - DMDNB as marker for plastic explosives (peroxidases)
 - HNS for ejection systems (haloperoxidases, biomimetics)
 - BT as EM precursor (hydroxylases, biomimetics)
3. DMDNB results
4. HNS results (preliminary)
5. BT results (biomimetic catalyst development)
6. Present, Near-Term, Far-Term Strategies for Green EM Synthesis
7. Recent Examples of Near-Term Enzyme Engineering Successes

CONVENTIONAL vs GREEN PROCESSING

Hypothetical example of C-1 oxidation of D-Glucose

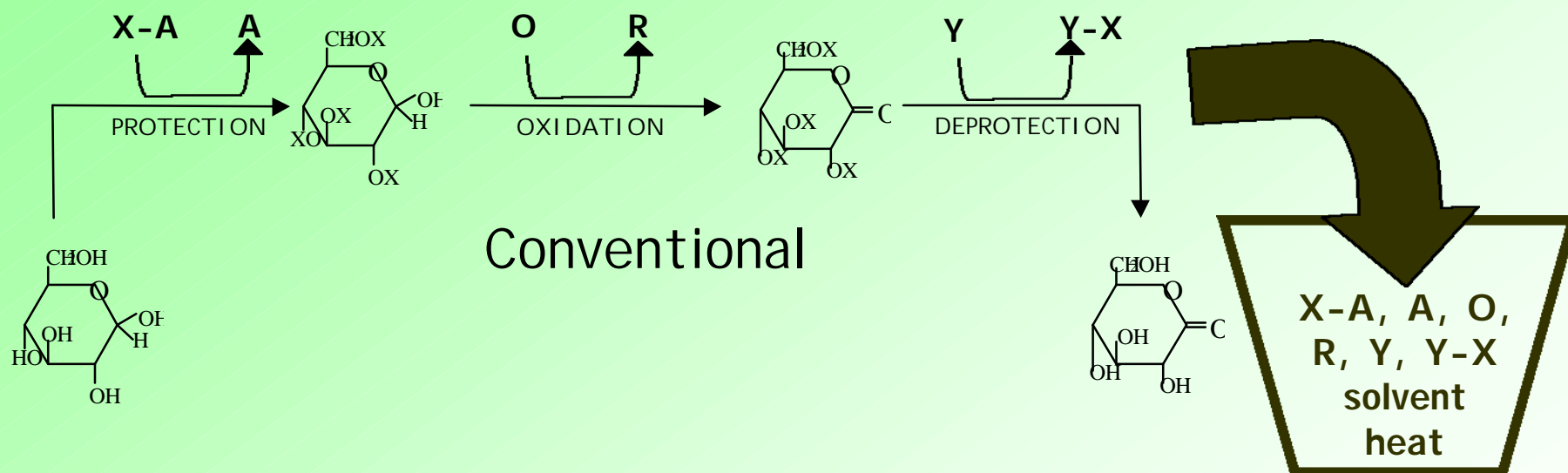


Issues are:

- Waste
- Heat
- Solvents

CONVENTIONAL vs. GREEN PROCESSING

Hypothetical example of C-1 oxidation of D-Glucose

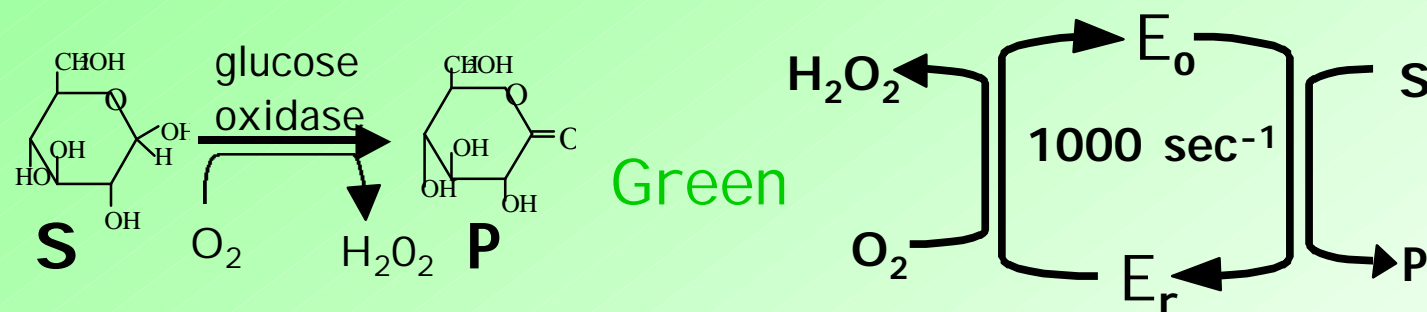


Strategy dominated by protection/deprotection (p/d)

- p/d required because of lack of specificity (catalysts/reactions)
- p/d creates *waste*
- inefficient (or no) catalysts require *high temperatures, solvents*

CONVENTIONAL vs. GREEN PROCESSING

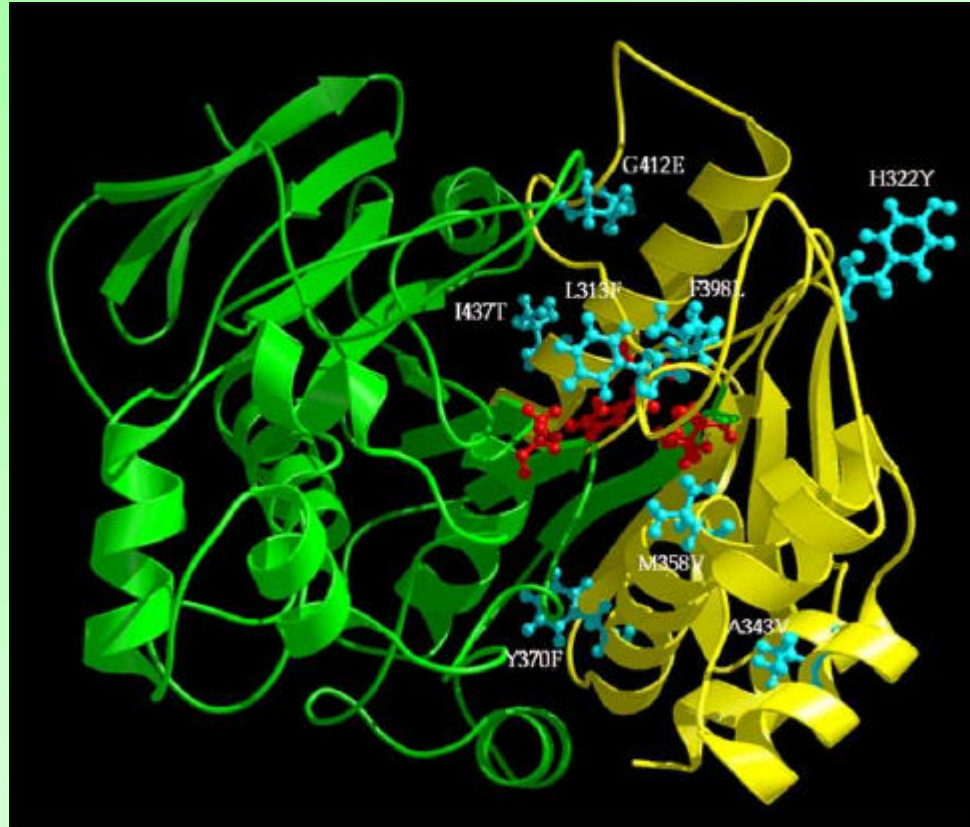
Hypothetical example of C-1 oxidation of D-Glucose



Enzymes have evolved biomolecular recognition to achieve:

- regioselectivity
 - stereoselectivity
 - reaction selectivity
 - low barriers/fast catalysis
 - active site dielectric control
- } no p/d !
- } no high temp/pressure
- } no toxic solvents

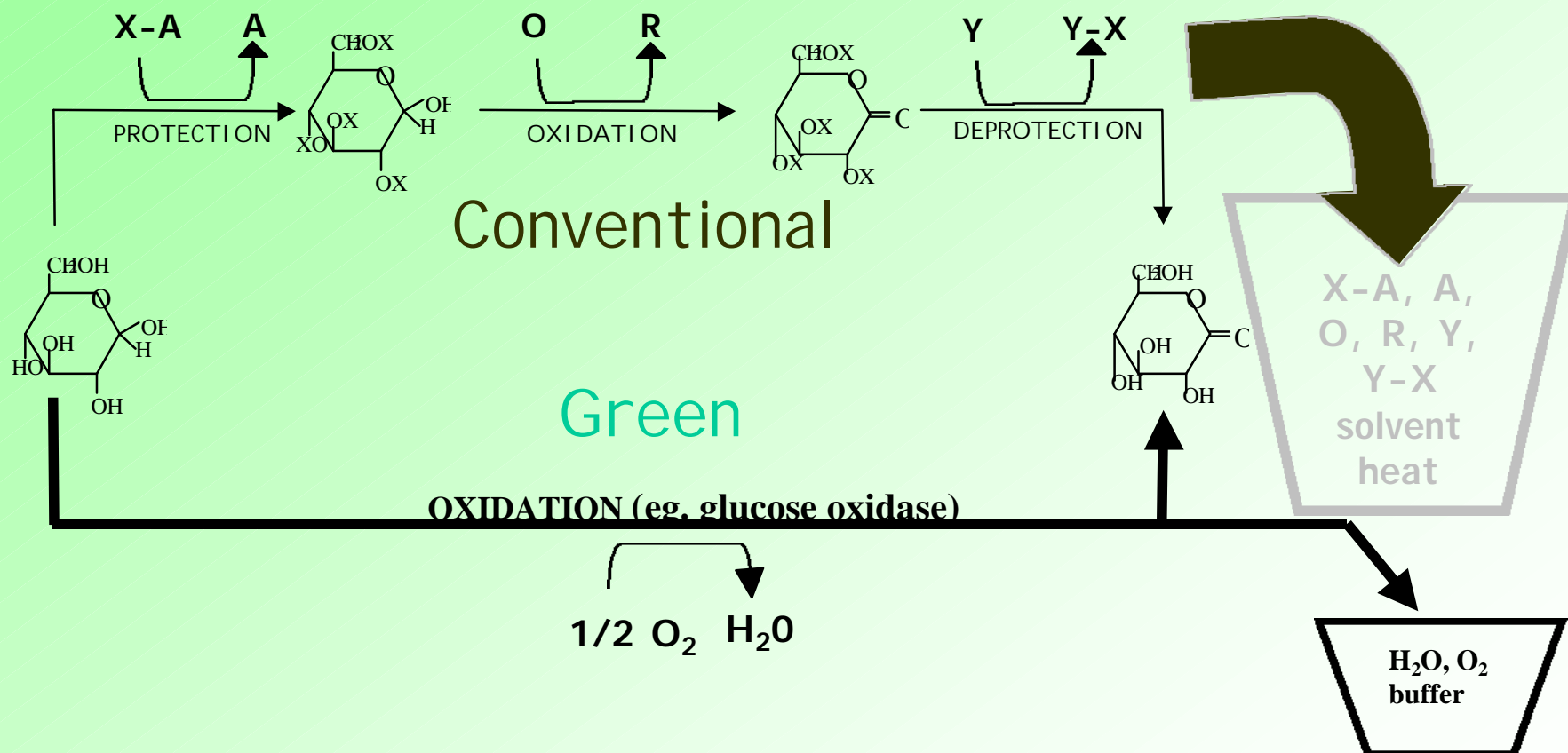
Green Processing



An enzyme

WASTE COMPARISONS, CONVENTIONAL vs GREEN PROCESSING

Hypothetical example of C-1 oxidation of D-Glucose



INITIAL EM TARGETS FOR GREEN SYNTHESIS

DMDNB

(10 lb waste/lb)

(2,3-dimethyl-2,3-dinitrobutane)
marker for plastic explosives

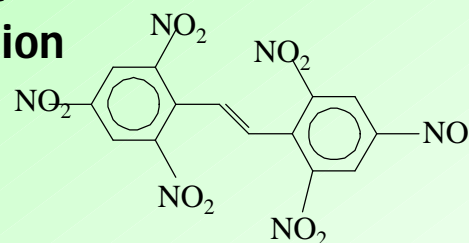


PEROXIDASES

HNS

(80 lb waste/lb)

(2,2',4,4',6,6'-hexanitrostilbene) heat resistant EM for ejection systems, other

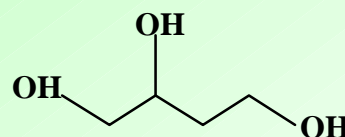


**HALOPEROXIDASES
MESOPOROUS
BIOMIMETICS**

BT

(13 lb waste /lb)

(1,2,4-butanetriol)
BTTN precursor,
plasticizer

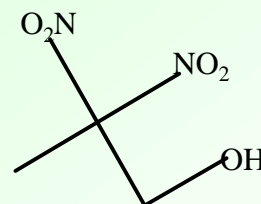


**PORPHYRIN
BIOMIMETICS**

DNPOH

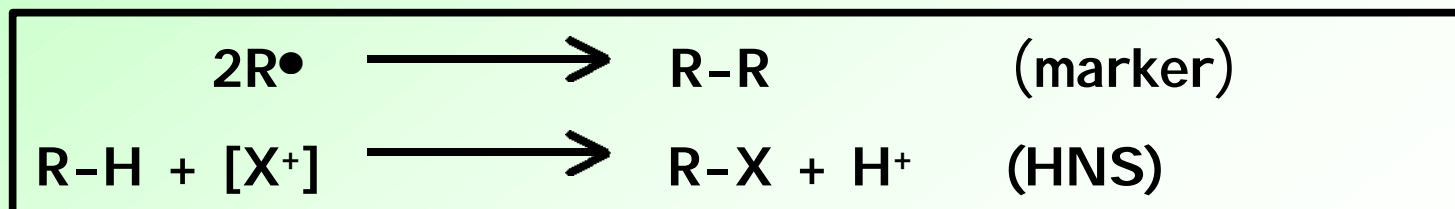
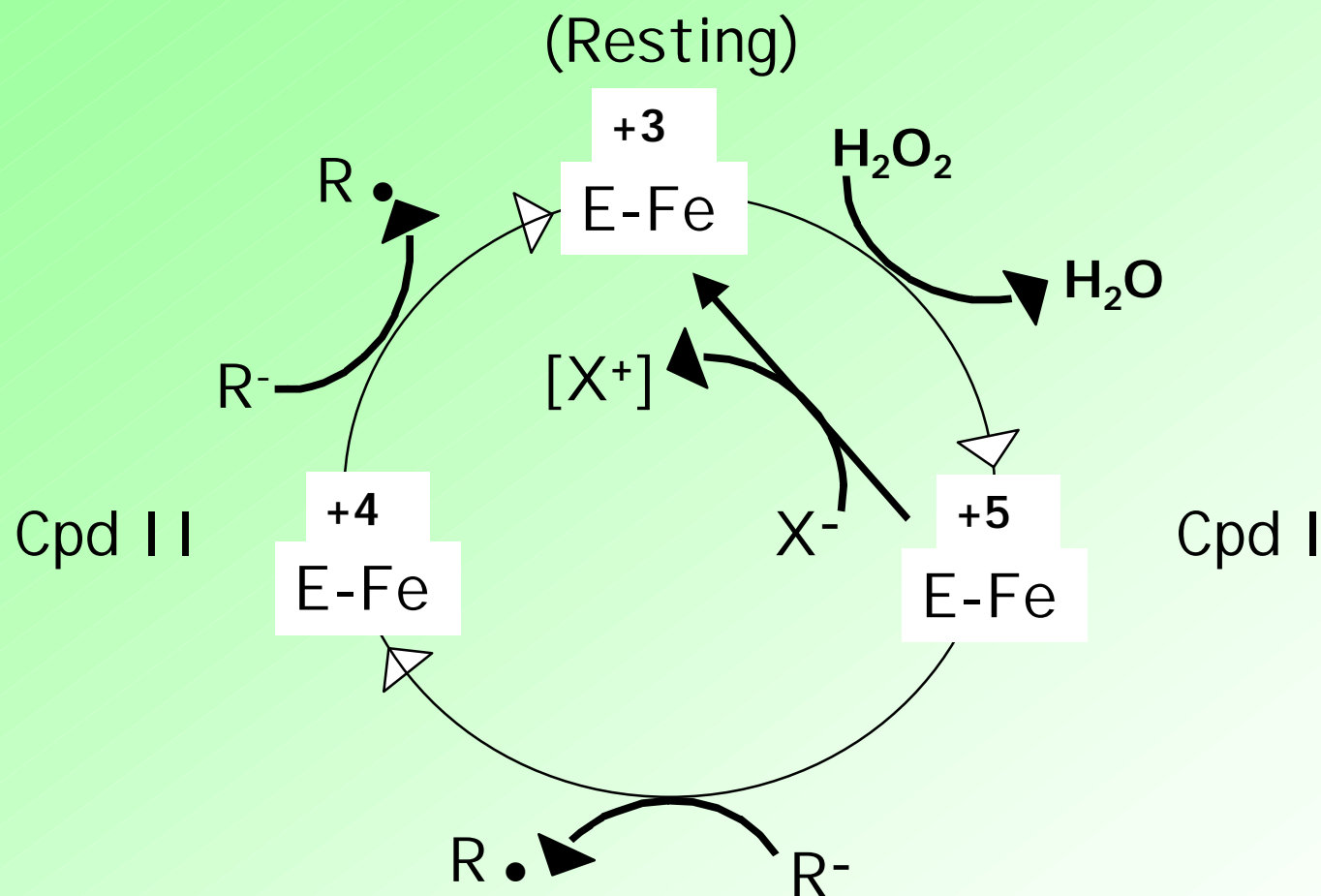
(19 lb waste/lb)

(2,2,-dinitropropanol)
plasticizer precursor,
other



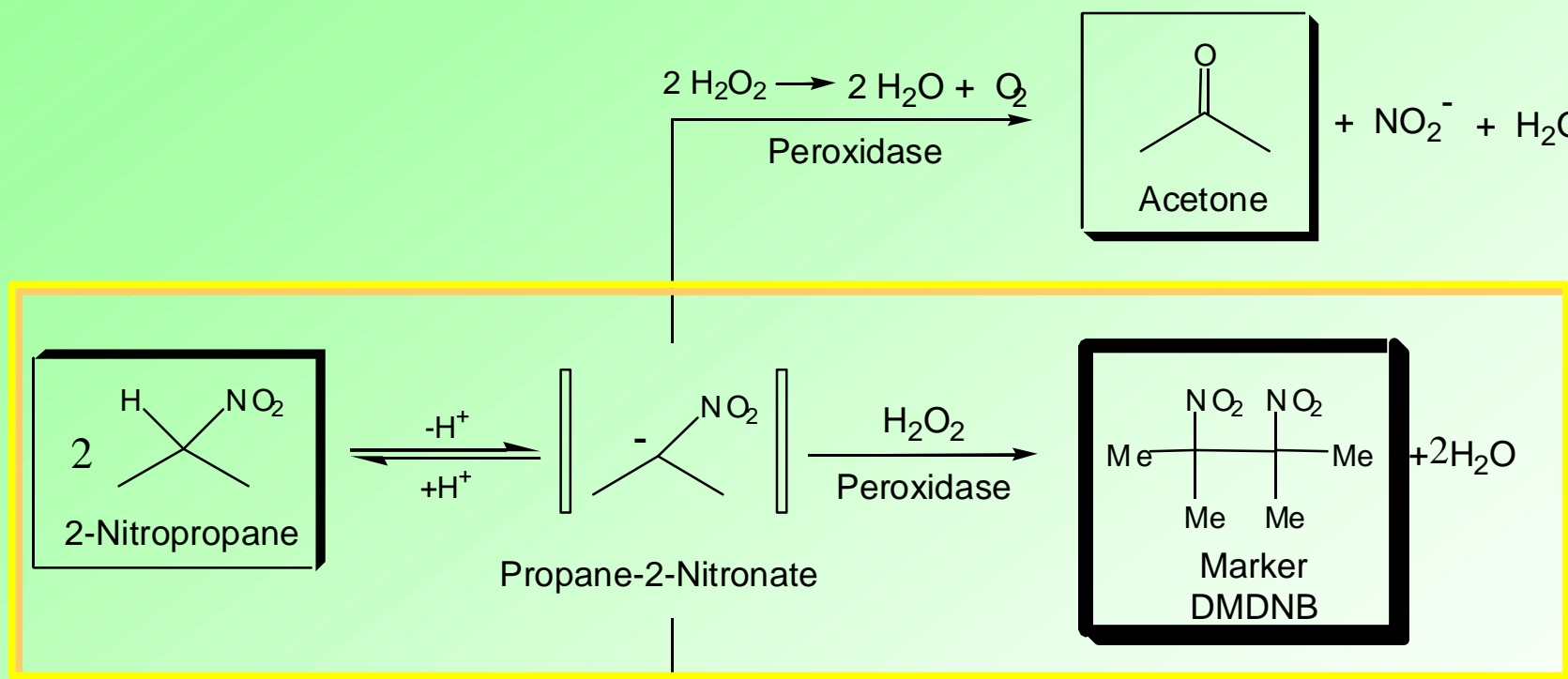
PEROXIDASES

Peroxidase Mechanism is Basis of Marker and HNS Projects

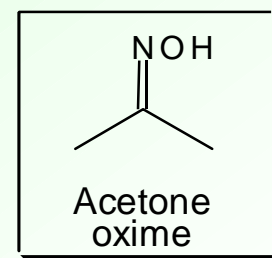


Green Synthesis of DMDNB Marker

(Based on D.J.T. Porter and H.J. Bright, J. Biol. Chem. 258, 9913-9924 (1983))

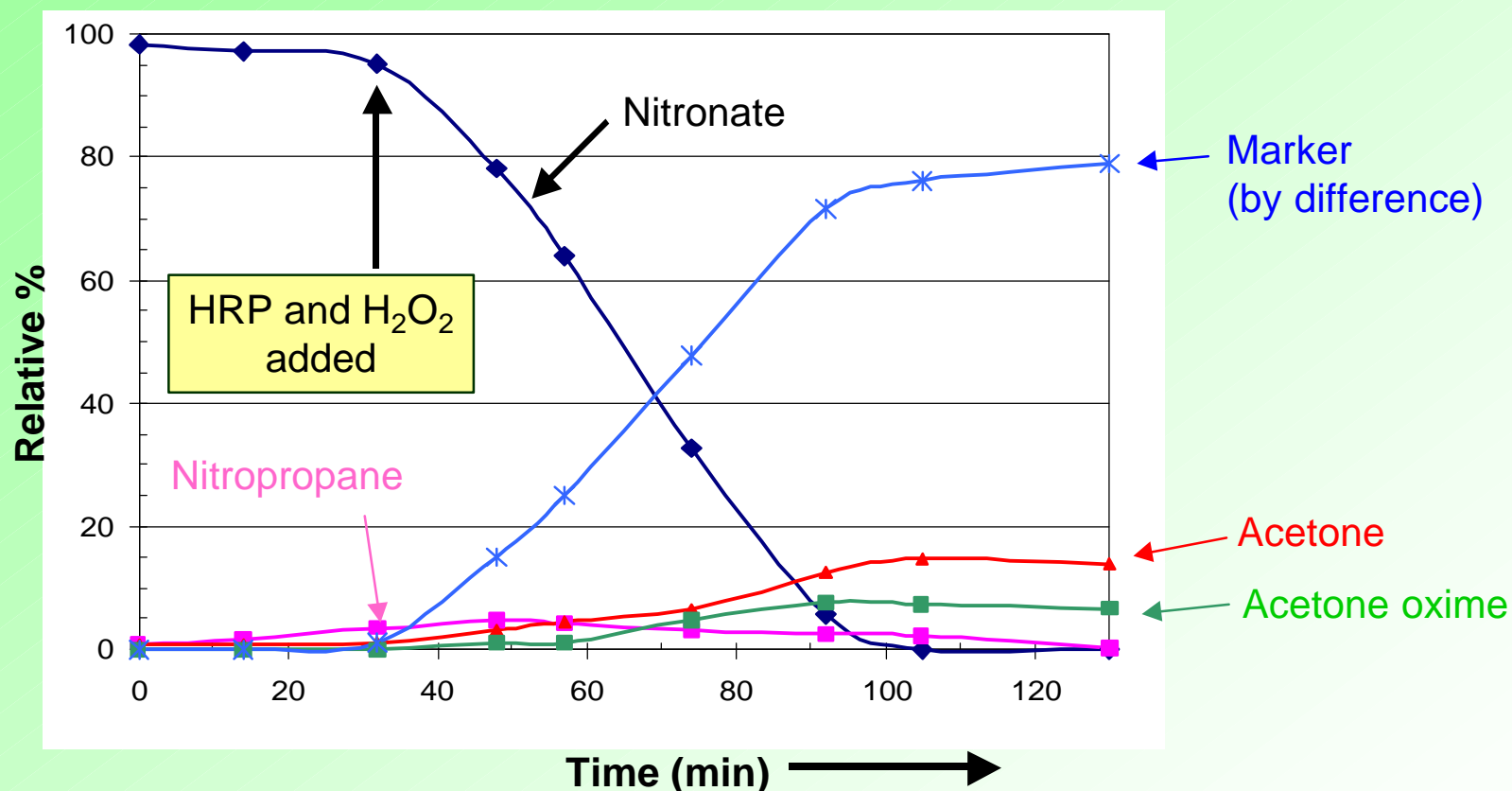


- VERY GREEN !**
 Solvent is 100% Water
 Only other product will be water
 Marker crystallizes during reaction



J. Kenar, NSWCIH, 1999

Time Course of Enzyme-Catalyzed Synthesis of Marker (By ^1H NMR) ~1670 Turnovers of Horseradish Peroxidase (HRP)



Conditions: Aqueous, pH 6.3, 25° C

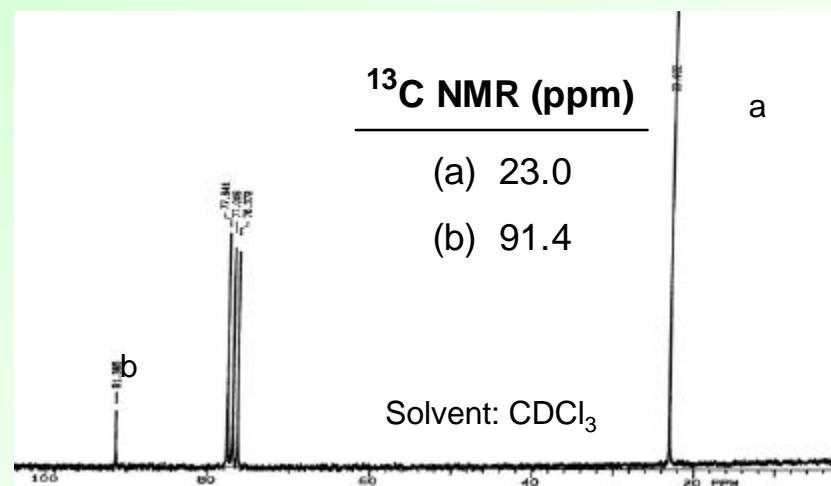
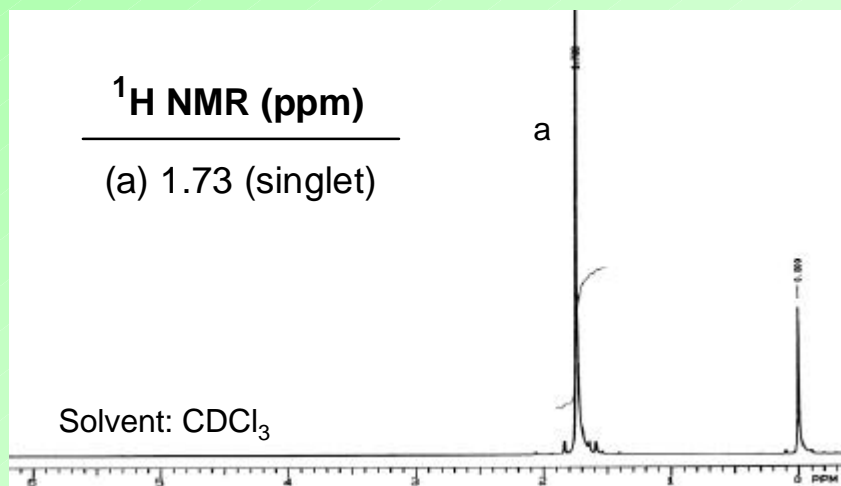
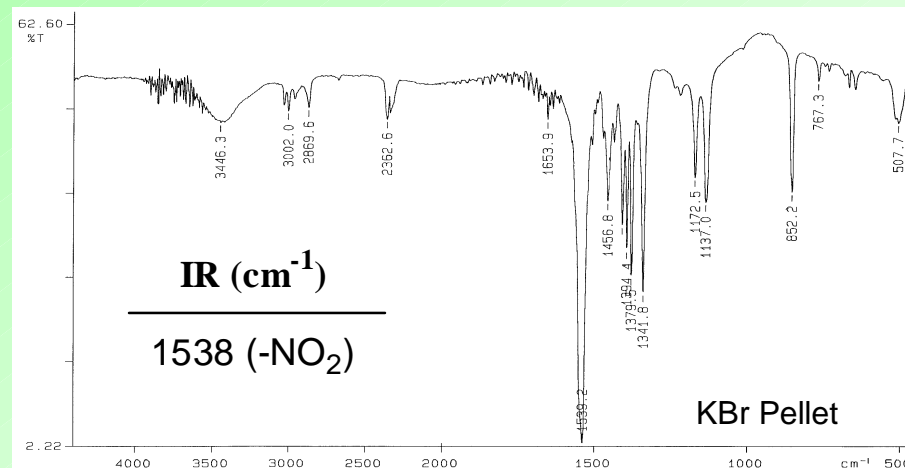
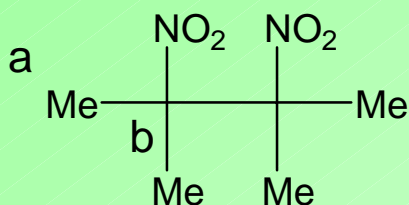
[Nitronate] = 100 mM

[H_2O_2] = 120 mM

[HRP] = 60 μM

J. Kenar, NSWCIH, 1999

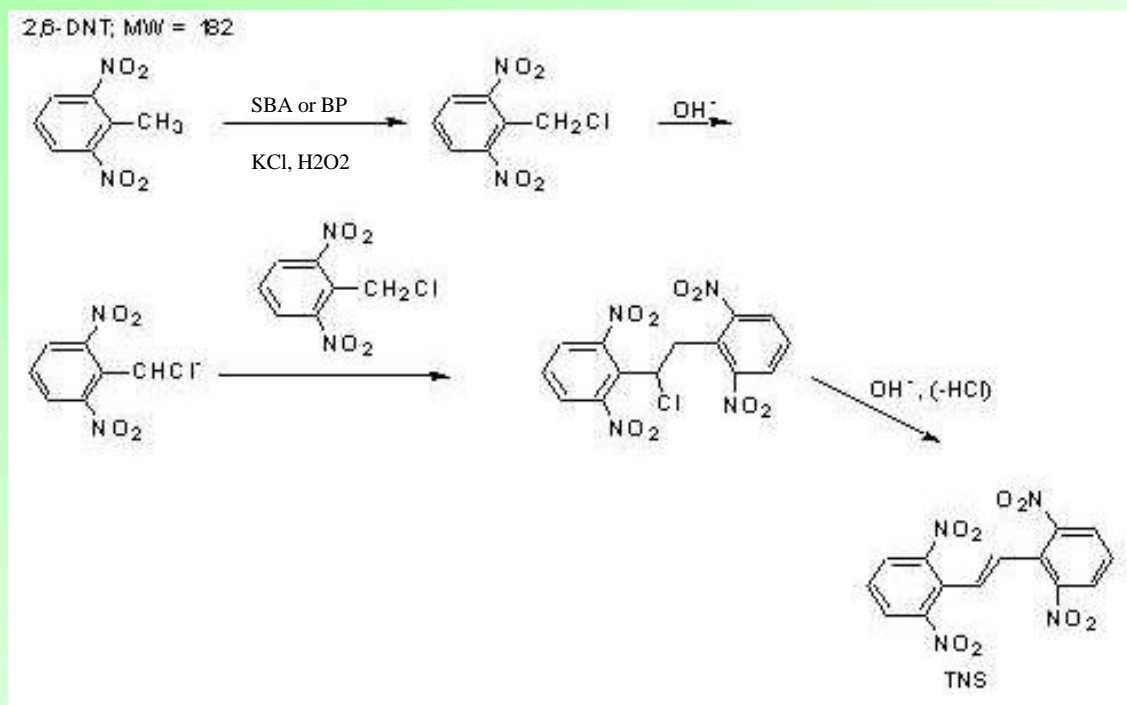
Marker From Enzyme-Catalyzed Reaction is Identical to Authentic Marker



J. Kenar, NSWCIH, 1999

Green Synthesis of HNS

Model Halogenation of 2,6 DNT Catalyzed by
Bromoperoxidase (BP) and by BP Mimics

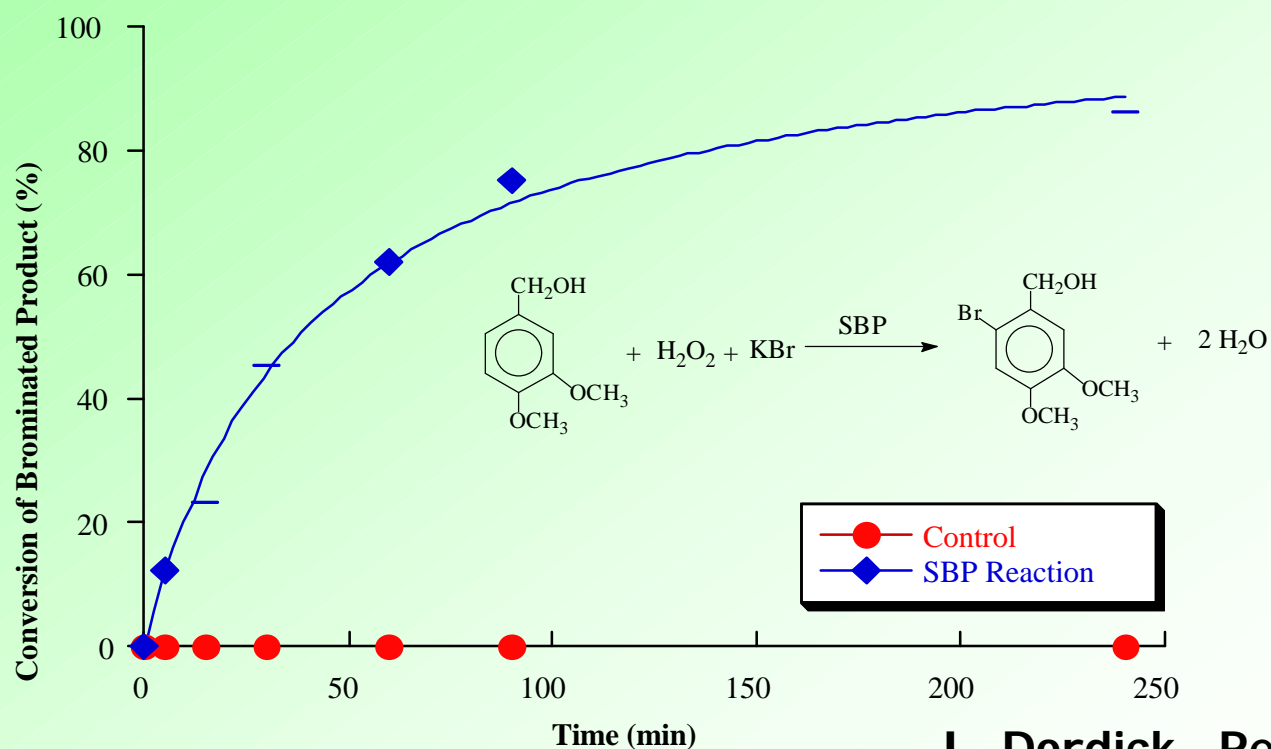


A. Butler, UCSB 1999

Green Synthesis of HNS

Model Halogenation by Soybean Peroxidase (SBP)

- time course for bromination of veratryl alcohol catalyzed by SBP (but wrong product!)

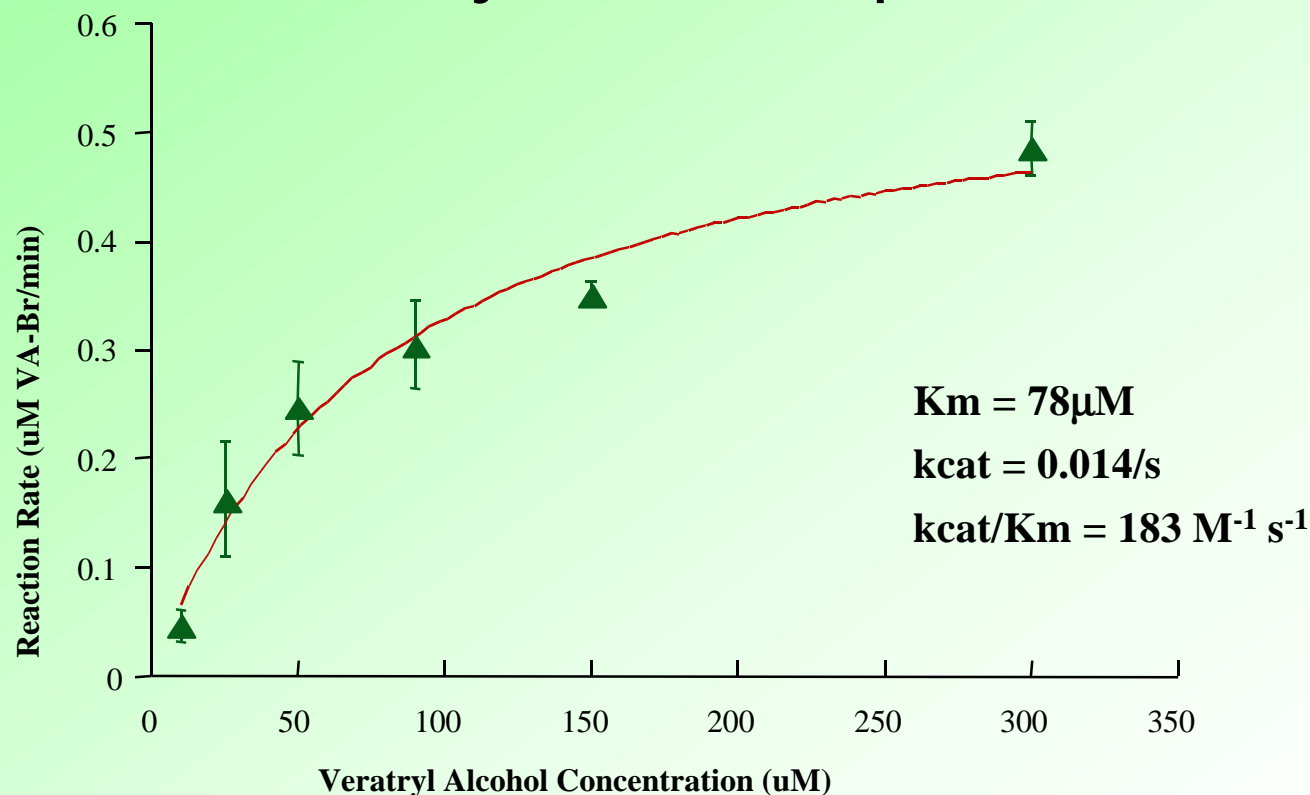


J. Dordick, Rennselaer 1999

Green Synthesis of HNS

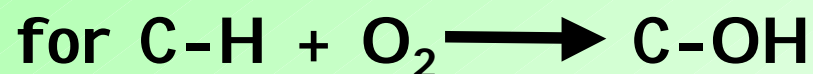
Model Halogenation by Soybean Peroxidase (SBP)

- steady state kinetic parameters



Green Synthesis of BT

Synthetic Porphyrin Mimics of Hydroxylase Enzymes

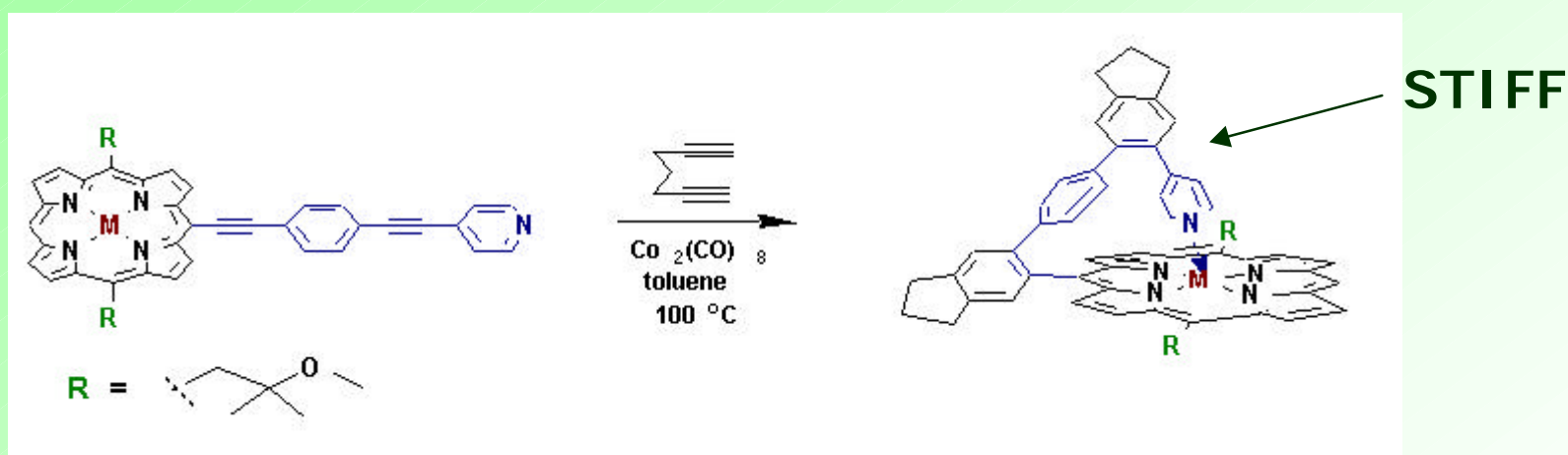


- Critical high-potential enzyme intermediate is hexacoordinate $\text{Fe}^{\text{IV}} = \text{O}$ porphyrin (like Cpd I in HRP)
- So precursor to this intermediate in mimic must be pentacoordinate and the fifth ligand must be rigidly held to keep Fe in planar (non-bowed) high potential state
- There has been progress on both fronts

M. Therien, U. Penn, 1999

Green Synthesis of BT

Synthetic Porphyrin Mimics of Hydroxylase Enzymes



- Metal ligand bond length remains fixed regardless of metal oxidation state or nature of the bonding interactions on the opposite face of the porphyrin

M. Therien, U. Penn, 1999

GREEN SYNTHESIS OF ENERGETIC MATERIALS

CURRENT MIX 'N MATCH PROBLEMS

- Nature's (ots) enzymes were not evolved with molecular recognition for EM synthesis in mind !
- therefore, only non-specific ots enzymes (e.g. free radical chain initiators like [halo]peroxidases) can be matched to EM targets

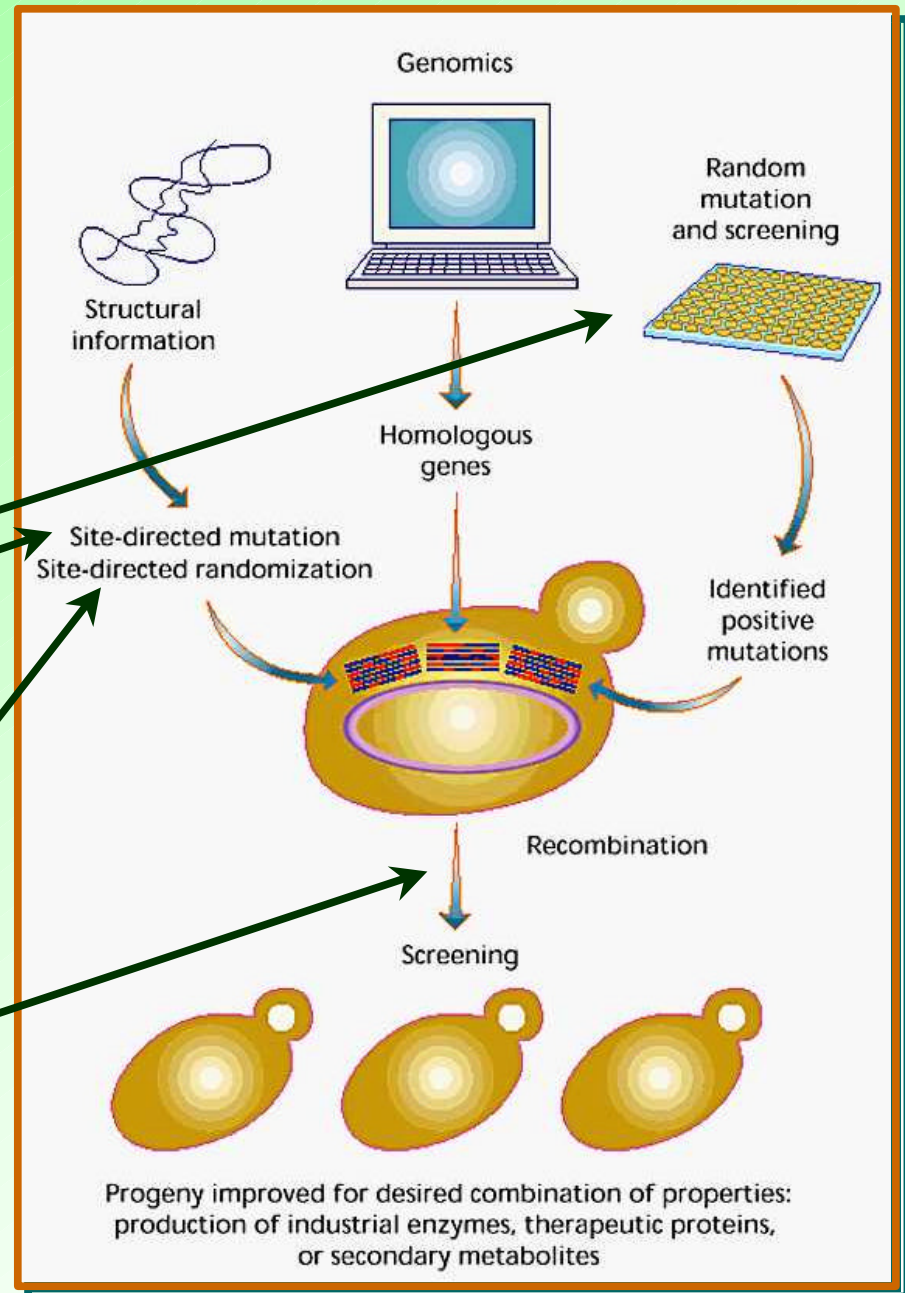
NEAR/FAR-TERM SOLUTIONS

- | | | | |
|------|---|---|--|
| Near | { | - | Design new schemes to exploit enzymes/biomimetics |
| Term | | - | Engineer range of rugged enzymes to recognize EM targets and catalyze fast reactions |
| Far | { | - | Mobilize full range of evolutionary, computational and bioinformatics technologies |
| Term | | - | EM synthesis in plants (Metabolic Engineering) |

Engineered Enzymes for Next Phase of Green EM Processing

Enzyme Engineering Tools:

- Random Mutation screening (RMS)
- site-directed mutation (SDM)
- site-directed randomization (SDR)
- directed molecular evolution (DME)



Recent Enzyme Engineering Advances Using Directed Molecular Evolution

April 1999 Nature Biotechnology: Cherry et al. (NOVO BIOTECH)

EM-relevant \longrightarrow Directed Evolution of Fungal Peroxidase

- improved thermal stability 174x
- improved oxidative (H_2O_2) stability 100x

THESE RESULTS VINDICATE ONR DECISION TO ENGINEER REDOX ENZYMES

April 12th Chem. and Eng. News, **ONR PI** Frances Arnold (CALTECH)

Hydrolases for penicillin production

- 100x more active than WT in organic solvents
- 18° C more stable, and more active, than WT

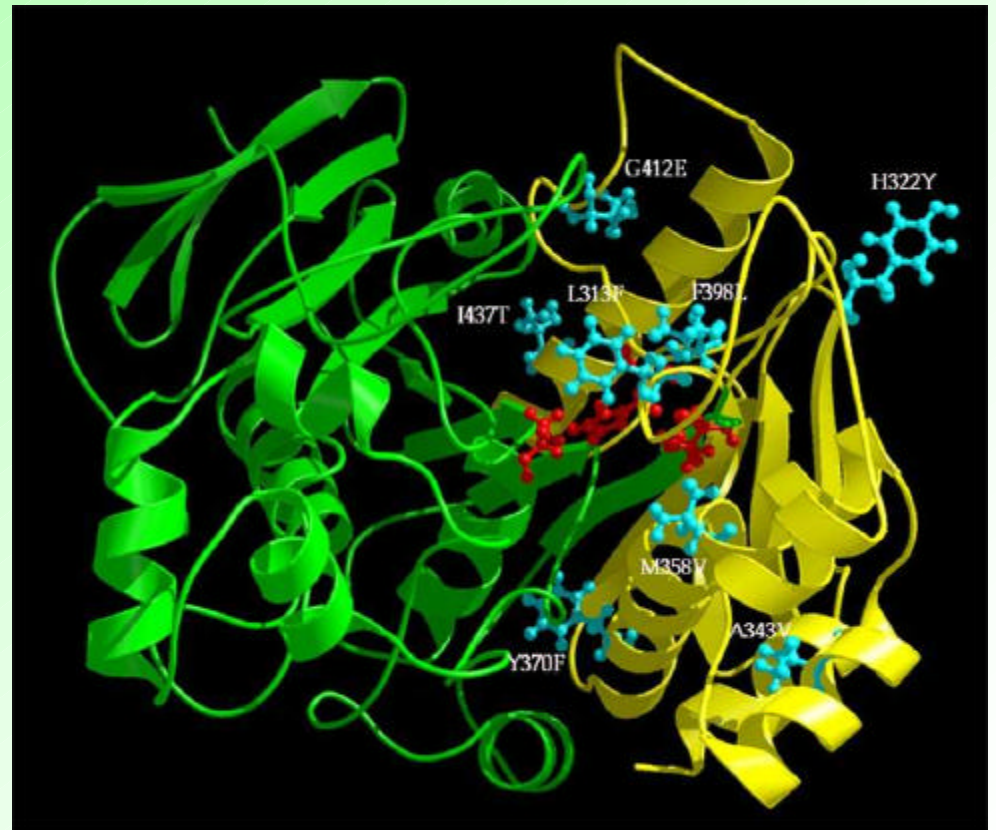
EM-relevant \longrightarrow Hydroxylase (P450) using H_2O_2

- 11x more active than WT in one cycle

ROOM TEMPERATURE HYDROXYLATION ON CARBON IS HOLY GRAIL

Structure of Test Tube-Evolved Enzyme

- ◆ Hydrolase required to be thermally toughened for commercial antibiotic synthesis
- ◆ Random accumulation of mutations (colored residues) in elevated temperature screens
- ◆ after 6 generations enzyme is thermostabilized by 18°C (and more active than WT!)



F. Arnold 1998